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Morphological and molecular evidence support a new species of the genus *Leptobrachella* (Anura, Megophryidae) from central China

Liming Jiang[1,2], Jing Liu[3], Yun Huang[1], Hongyan Zhang[1,2], Bin Wang[1,2]

1 *Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610213, Sichuan, China*
2 *University of Chinese Academy of Sciences, Beijing 100049, China*
3 *College of Resources and Environment, Moutai Institute, 564500, Renhuai, China*

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Corresponding author: Bin Wang (wangbin@cib.ac.cn)

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Abstract

Based on morphological comparisons and molecular phylogenetic analyses, a new species of the genus *Leptobrachella* from Xinshao County, Hunan Province, China, is described. Phylogenetic analyses of mitochondrial 16S rRNA and COI gene sequences support the new species as an independent lineage. The uncorrected p-distances of the 16S rRNA and COI genes between the new species and its closest congeners are 2.1% and 8.1%, respectively. The new species is distinguished from its congeners by a combination of the following characters: (1) SVL 27.8–31.4 mm in males; (2) toes rudimentarily webbed, with narrow lateral fringes; (3) tympanum distinct, surrounded by distinct tubercles; (4) dorsal surface shagreened, with small, raised tubercles and longitudinal ridges; (5) ventral surface creamy white, with dark brown spots on the chest and margins; (6) distinct black spots present on the flanks; (7) heels not meeting when hindlimbs are flexed at right angles to the body axis; and (8) tibia–tarsal articulation reaching between the eye and the tympanum.

Key Words

Leptobrachella, molecular phylogenetic analysis, morphology, new species

Introduction

The genus *Leptobrachella* Smith, 1925, comprises Asian leaf-litter frogs that are widely distributed across southern China, northeastern India, Myanmar, Indochina, the Malay Peninsula, and Borneo (Frost 2025). Species within this genus are small and predominantly inhabit the leaf litter near forest streams, exhibiting considerable ecological and morphological diversity. Although the taxonomic history of this group is complex, with members previously assigned to several genera, including the unavailable names *Paramegophrys* Liu, 1964, and *Carpophrys* Sichuan Biological Research Institute, 1977 (both nomina nuda), as well as *Leptolalax* Dubois, 1980, *Lalax* Delorme, Dubois, Grosjean & Ohler, 2006, and *Lalos* Dubois, Grosjean, Ohler, Adler & Zhao, 2010, recent integrated taxonomic studies based on phylogenetic analyses have clarified their systematic positions, unifying these names under *Leptobrachella* (Chen et al. 2018).

In recent years, the combined application of molecular systematics, morphological comparison, and bioacoustic analysis has led to the continual discovery of new *Leptobrachella* species, particularly in biodiversity hotspots of southern China, such as the Wuling Mountains, Nanling Mountains, and the Yunnan–Guizhou Plateau (Huang et al. 2025a; Liu et al. 2025; Luo et al. 2025; Wu et al. 2025). To date, 114 species have been described within this genus, four of which are definitively recorded in Hunan Province, China: *L. wulingensis* Qian, Xiao, Cao, Xia & Yang, 2020; *L. mangshanensis* (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang, 2018); *L. dong* Liu, Shi, Li, Zhang, Xiang, Wei & Wang, 2023; and *L. yongshunensis* Huang, Wu, Jiang & Zhang, 2025 (Huang et al. 2025a). However, due to their high morphological similarity and restricted distributions,

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it is suspected that numerous cryptic species remain undiscovered (Liu et al. 2023; Li et al. 2024; Luo et al. 2025).

Xinshao County is located in central-southern Hunan Province, China, in the transitional zone between the Xuefeng and Wuling Mountains. This area is characterized by complex topography and a well-developed river network, providing suitable habitats for *Leptobrachella*. During recent field surveys in this region, we collected a series of *Leptobrachella* specimens. Based on comprehensive morphological and molecular phylogenetic analyses, we identified this population as a new species, which is formally described herein.

Material and methods

Sampling

Three male specimens of the unnamed taxon were collected from Xinshao County, Hunan Province, China (Fig. 1). All animal procedures were performed according to the protocols approved by the Animal Care Advisory Committee of the Chengdu Institute of Biology, Chinese Academy of Sciences, China (permit number: CIB20191084). All specimens were fixed in 10% buffered formalin for 10 h and then transferred to 75% ethanol. Tissue samples were preserved separately in 95% ethanol prior to fixation. Specimens are deposited in the Chengdu Institute of Biology, Chinese Academy of Sciences (CIB, CAS), China.

Molecular data and phylogenetic analyses

DNA was extracted from muscle tissue using a DNA extraction kit from Tiangen Biotech Co., Ltd. (Beijing). Two fragments of the mitochondrial 16S rRNA (16S) and cytochrome c oxidase subunit I (COI) genes were amplified. For 16S, the primers P7 (5'-CGCCTGTTTACCAAAAACAT-3') and P8 (5'-CCGGTCTGAACTCAGATCACGT-3') were used (Simon et al. 1994), and for COI, Chmf4 (5'-TYTCWACWAAYCAYAAAGAYATCGG-3') and Chmr4 (5'-ACYTCRGGRTGRCCRAARAATCA-3') were used (Che et al. 2012). Gene fragments were amplified under the following conditions: an initial denaturing step at 95 °C for 4 min; 36 cycles of denaturing at 95 °C for 30 s, annealing at 52 °C (for 16S) or 47 °C (for COI) for 40 s, and extension at 72 °C for 70 s. Sequencing was conducted using an ABI3730 automated DNA sequencer by Shanghai DNA BioTechnologies Co., Ltd. (Shanghai, China). New sequences were deposited in GenBank (for GenBank accession numbers, see Table 1).

For phylogenetic analyses and genetic divergence analyses, we downloaded available corresponding sequence data for all related species from GenBank according to previous studies (Huang et al. 2025a; Luo et al. 2025; Table 1). Sequences were assembled and aligned using the ClustalW module in BioEdit v.7.0.9.0 (Hall 1999) with default settings.

Based on the concatenated 16S + COI dataset, phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) methods, implemented in PhyML 3.0 (Guindon et al. 2010) and MrBayes v.3.2 (Ronquist et al. 2012), respectively. The best-fit model (GTR + G + I) was selected using the Bayesian information criterion (BIC) in PartitionFinder 2 (Lanfear et al. 2012).

For the ML analysis, nodal support was assessed with 1000 bootstrap replicates. For the BI analysis, four Markov chains were run for 50 million generations, sampling every 1000 generations. Convergence was confirmed by ensuring that the average standard deviation of split frequencies fell below 0.01. The first 25% of sampled trees were discarded as burn-in, and a 50% majority-rule consensus tree was generated from the remaining trees to calculate Bayesian posterior probabilities.

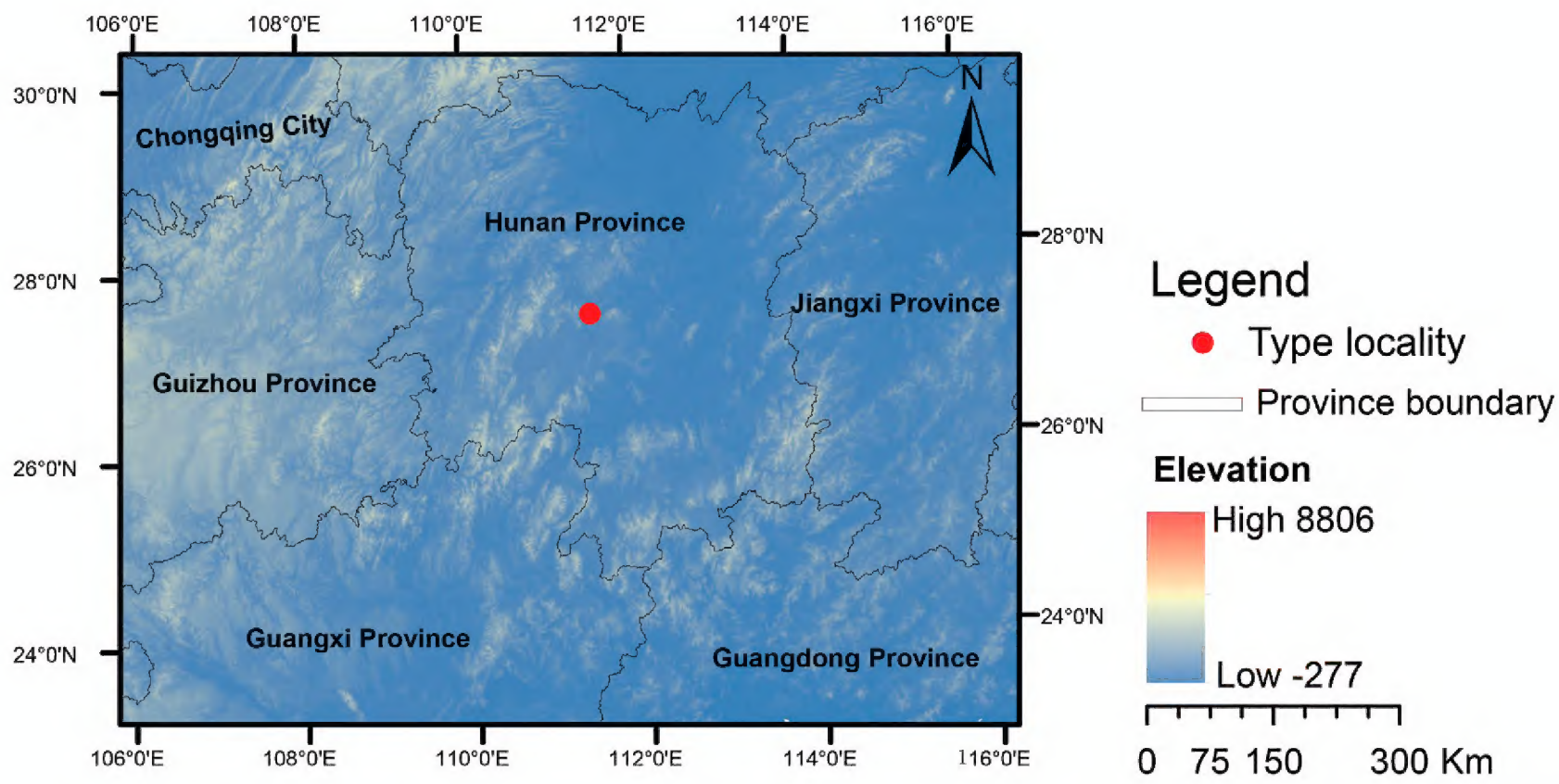

Figure 1. Map showing the distribution sites of *Leptobrachella xinshaoensis* sp. nov. The base maps are from the Standard Map Service website (http://bzdt.ch.mnr.gov.cn/index.html).

Table 1. Information on samples used in molecular phylogenetic analyses in this study.

Species	Voucher No.	Locality	GenBank No.	
			16S	COI
Leptobrachella xinshaoensis sp. nov.	CIB XS20240319001	Xinshao County,Hunan, China	PX856296	PX852802
Leptobrachella xinshaoensis sp. nov.	CIB XS20240319002	Xinshao County,Hunan, China	PX856297	PX852803
Leptobrachella xinshaoensis sp. nov.	CIB XS20240319003	Xinshao County,Hunan, China	PX856298	PX852804
Leptobrachella suiyangensis	GZNU 20180606002	Suiyang County, Guizhou, China	MK829648	/
Leptobrachella xishuiensis	GZNU20240726007	Xianyuan Town, Xishui County, Guizhou	PQ604660	/
Leptobrachella jinshaensis	CIBJS20200516005	Lengshuihe Nature Reserve, Jinsha County, Guizhou, China	OQ024816	OQ024417
Leptobrachella purpuraventra	CIB119072	Mount Baima, Qixingguan, Bijie, Guizhou, China	OQ024811	OQ024412
Leptobrachella jinyunensis	CIB119058	Mount Jinyun, Beibei District, Chongqing, China	OQ024797	OQ024396
Leptobrachella bijie	CIB119068	Mount Baima, Qixingguan, Bijie, Guizhou, China	OQ024807	OQ024406
Leptobrachella chishuiensis	CIBCS20190518047	Chishui National Nature Reserve, Chishui, Guizhou, China	MT117055	OQ024422
Leptobrachella niveimontis	KIZ 015734	Daxueshan Nature Reserve, Yunnan, China	MT302618	/
Leptobrachella yunyangensis	GZNU 20210629001	Lianhua Village, Yunyang County, Chongqing, China	OL800366	/
Leptobrachella dong	CIBZNY2022002	Suining County, Hunan Province, China	OP764539	PP061400
Leptobrachella dushanensis	CIBDS20230310001	Dushan County, GuizhouProvince, China	PP061390	PP061404
Leptobrachella bourreti	VNMN 010073	Bat Xat District,Lao Cai, Vietnam	PP061393	PP061407
Leptobrachella yeae	KIZ025778	Mount Emei, Sichuan, China	KX811928	KX812166
Leptobrachella graminicola	TOLO2022.518	Mount Pu Ta Leng,Lao Cai, Vietnam	PP061392	PP061406
Leptobrachella wulingensis	JWS2024422	Zhangjiajie Froest National Park, Hunan Province, China	PQ222386	PQ222459
Leptobrachella wulingensis	JWS2024424	Zhangjiajie Froest National Park, Hunan Province, China	PQ222387	PQ222460
Leptobrachella wulingensis	JWS2024426	Zhangjiajie Froest National Park, Hunan Province, China	PQ222388	PQ222461
Leptobrachella wulingensis	CSUFT194	Tianquanshan Forest Park, Zhangjiajie, Hunan Province, China	MT530316	/
Leptobrachella yongshunensis	HJ2024001	Hunan Xiaoxi National Nature Reserve, Hunan Province, China	PQ227124	PQ227123
Leptobrachella yongshunensis	HJ2024004	Hunan Xiaoxi National Nature Reserve, Hunan Province, China	PQ227127	PQ227118
Leptobrachella yongshunensis	HJ2024005	Hunan Xiaoxi National Nature Reserve, Hunan Province, China	PQ227128	PQ227119
Leptobrachella dorsospina	SYS a004974	Yushe Forest Park, Shuicheng, Guizhou, China	MW046197	/
Leptobrachella purpurus	SYS a006531	Jinzhuzhai Village, Yunnan, China	MG520355	/
Leptobrachella alpina	KIZ 046816	Huangcaoling, Yunnan, China	MH055866	/
Leptobrachella eos	NCSM 80551	Boun Tay, Phongsaly, Laos	MH055887	/
Leptobrachella oshanensis	CIB EMS20190422SSG1-3	Shengshuige, Mount Emei, Sichuan, China	MT957032	/
Leptobrachella korifi	KUHE 19134	Doi Inthanon, Chiang Mai, Thailand	LC741033	/
Leptobrachella sinorensis	KUHE 19809	Mae Hong Son, Thailand	LC741034	/
Leptobrachella murphyi	KIZ 034039	Doi Inthanon, Chiang Mai, Thailand	MZ710519	/
Leptobrachella tengchongensis	SYS a003766	Gaoligong Shan, Tengchong County, Yunnan, China	MH055897	/
Leptobrachella mangshanensis	MSZTC 201703	Mangshan, Hunan, China	MG132198	/
Leptobrachella liui	ZYCA907	Wuyi Shan, Fujian, China	MH055908	/
Leptobrachella verrucosa	GEP a062	Lianshan Bijiashan Nature Reserve, Lianshan County, Guangdong, China	OP279592	/
Leptobrachella dayaoshanensis	NNU202103018	Dayaoshan, Jinxiu County, Guangxi, China	PQ476281	/
Leptobrachella yunkaiensis	SYS a004663	Dawuling Forest Station, Guangdong, China	MH605584	/

Species	Voucher No.	Locality	GenBank No.	
			16S	COI
Leptobrachella kungfu	GEP a419	Laoxiangshan, Guangdong, China	PX431625	/
Leptobrachella shimentaina	SYS a007684	Shimentai Nature Reserve, Yingde City, Guangdong, China	ON017330	/
Leptobrachella laui	SYS a002450	Shenzhen, Guangdong, China	MH055904	/
Leptobrachella bashaensis	GIB196404	Basha Nature Reserve, Guizhou, China	MW136295	/
Leptobrachella maoershanensis	KIZ 07614	Mao'er Shan, Guangxi, China	MH055927	/
Leptobrachella flaviglandulosa	KIZ 016065	Xiaoqiaogou Nature Reserve, Yunnan, China	MT302625	/
Leptobrachella phiaoacensis	IEBR A.5195	Phia Oac-Phia Den NP, Cao Bang, Vietnam	OR405871	/
Leptobrachella khasiorum	SDBDU 2009.329	Khasi Hills, Meghalaya, India	KY022303	/
Leptolalax tamdil	MZMU2225	NA	MW665131	/
Leptobrachella yingjiangensis	SYS a006535	Jinzhuzhai Village, Yunnan, China	MG520357	/
Leptobrachella puhoatensis	AMS R184852	Pu Hoat Nature Reserve, Nghe An, Vietnam	KY849588	/
Leptobrachella petrops	ROM 13483	Ba Vi National Park, Ha Tay, Vietnam	MH055901	/
Leptobrachella namdongensis	VNUF A.2017.37	Thanh Hoa, Vietnam	MK965389	/
Leptobrachella ventripunctata	KIZ 020421	Jinuo Shan, Yunnan, China	MH055825	/
Leptobrachella guinanensis	NNU 00557	Shangsi County, Guangxi, China	OP548561	/
Leptobrachella feii	KIZ 048894	Xiaoqiaogou Nature Reserve, Yunnan, China	MT302634	/
Leptobrachella aspera	SYS a007744	Huanglianshan Nature Reserve, Lyuchun, Yunnan, China	MW046200	/
Leptobrachella pelodytoides	TZ819	NA	AF285192	/
Leptobrachella aerea	NCSM 76038	Vilabuly, Savannakhet, Laos	MH055809	/
Leptobrachella minima	K3124	Mae Lao Mae Sae, Doi Chiang Dao, Thailand	JN848369	/
Leptobrachella nyx	AMNH A163810	Mount Tay Conn Linh, Ha Giang, Vietnam	DQ283381	/
Leptobrachella shangsiensis	NHMG 1401032	Guangxi, China	MK095460	/
Leptobrachella phiadenensis	IEBR A.5206	Phia Oac-Phia Den NP, Cao Bang, Vietnam	OR405873	/
Leptobrachella pluvialis	MNHN 1999.5675	Sapa, Vietnam	JN848391	/
Leptobrachella nahangensis	ROM 7035	Na Hang Nature Reserve, Tuyen Quang, Vietnam	MH055853	/
Leptobrachella damingshanensis	NNU 202103281	Damingshan National Nature Reserve, Wuming County, Guangxi, China	MZ145229	/
Leptobrachella wumingensis	NNU 01058	Wuming County, Guangxi, China	OR194551	/
Leptobrachella yachangensis	NHMG 250401	Yachang Orchid National Nature Reserve, Leye, Guangxi, China	PX307240	/
Leptobrachella batxatensis	IEBR A.5223	Vietnam, Lao Cai Province, Bat Xat District	PP826339	/
Leptobrachella shiwandashanensis	NNU 202103213	Golden Camellia National Nature Reserve, Guangxi, China	MZ326692	/
Leptobrachella darongshanensis	NNU001152	Darongshan Nature Reserve, Guangxi, China	PX480157	
Leptobrachella wuhuangmontis	SYS a003485	Mt. Wuhuang, Pubei County, Guangxi, China	MH605577	/
Leptobrachella sungi	ROM 20236	Tam Dao, Vinh Phuc, Vietnam	MH055858	/
Leptobrachella zhangyapingi	KIZ 07258	Chiang Mai, Thailand	MH055864	/
Leptobrachella firthi	AMS R 176506	Ngoc Linh Nature Reserve, Kon Tum, Vietnam	JQ739207	/
Leptobrachella isos	AMS R 176469	Gia Lai, Vietnam	KT824767	/
Leptobrachella neangi	CBC 1609	Phnom Samkos Wildlife Sanctuary, Veal Veng District, Pursat, Cambodia	MT644613	/
Leptobrachella duyenae	IEBR A.5229	Vietnam, Khanh Hoa Province, Dien Khanh District	PP826345	/
Leptobrachella heteropus	KUHE 15487	Peninsula, Malaysia	AB530453	/
Leptobrachella sola	KU RMB20973	Malaysia	MH055973	/
Leptobrachella lateralis	IASST AR87	India	KU674834	/
Megophrys glandulosa	KIZ048439	Yunnan Province, China	KX811762	KX812165
Leptobrachium huashen	KIZ049025	Yunnan Province, China	KX811931	KX812075

Finally, uncorrected *p*-distances for the 16S and COI genes were calculated separately with 1000 bootstrap replicates in MEGA v.6.06 (Tamura et al. 2013).

Morphological comparisons

Morphological measurements were made with dial calipers to the nearest 0.1 mm by Liming Jiang, following Fei et al. (2009) and Huang et al. (2025a). Eighteen morphometric characters of eight adult specimens were measured (Suppl. material 1) and abbreviated as follows: ED (eye diameter, distance from the anterior corner to the posterior corner of the eye), FL (foot length, distance from the tarsus to the tip of the fourth toe), HDL (head length, distance from the tip of the snout to the articulation of the jaw), HDW (maximum head width, greatest width between the left and right articulations of the jaw), HLL (hindlimb length, distance from the tip of the fourth toe to the vent), IND (internasal distance, minimum distance between the inner margins of the external nares), IOD (interorbital distance, minimum distance between the inner edges of the upper eyelids), LAL (length of the lower arm and hand, distance from the elbow to the distal end of finger IV), LW (lower arm width, maximum width of the lower arm), ML (manus length, distance from the tip of the third digit to the proximal edge of the inner palmar tubercle), TFL (length of the foot and tarsus, distance from the tibiotarsal articulation to the distal end of toe IV), THL (thigh length, distance from the vent to the knee), TL (tibia length, distance from the knee to the tarsus), TW (maximum tibia width), TYD (maximum tympanum diameter), SL (snout length, distance from the tip of the snout to the anterior corner of the eye), SVL (snout–vent length, distance from the tip of the snout to the posterior edge of the vent), and UEW (upper eyelid width, greatest width of the upper eyelid margins measured perpendicular to the anterior–posterior axis).

Sex was determined by examination of primary sexual characteristics after dissection. Additional morphological data used for comparisons were obtained from the literature (Table 2).

Results

Phylogenetic analyses

A total of 528 base pairs (bp) of the 16S gene and 612 bp of the COI gene were concatenated. Maximum likelihood (ML) and Bayesian inference (BI) trees based on the mitochondrial DNA dataset showed nearly consistent topologies, although relationships among many lineages remained unresolved (Fig. 2), with strong support for the major nodes in both analyses. In both ML and BI trees, all samples of the genus *Leptobrachella* from this study clustered within a single group, forming a distinct lineage with high nodal support (BPP = 1.00; BS = 100%). All samples of the undescribed species clustered into one clade, which was deeply nested within the *Leptobrachella* clade and appeared to be sister to a clade comprising *L. wulingensis* and *L. yongshunensis*, with high support (BPP = 1.00; BS = 100%) (Fig. 2). The smallest pairwise genetic divergence between the undescribed species and its congeners was 2.1% (vs. *L. wulingensis*) for 16S and 8.1% (vs. *L. yongshunensis*) for COI. These values are higher than or comparable to those between some recognized species pairs, such as *L. bijie* and *L. jinshaensis*, and *L. dong* and *L. dushanensis* (Suppl. materials 1, 2).

Furthermore, these specimens are distinguishable from other congeners within the genus by a unique combination of morphological characters (see taxonomic account below).

Morphological comparisons

Comparative morphological data of the undescribed taxon and 83 recognized *Leptobrachella* species occurring north of the Isthmus of Kra are listed in Suppl. material 3. From the 27 congeners of the genus *Leptobrachella* occurring south of the Isthmus of Kra, by the presence of supra-axillary and ventrolateral glands, the undescribed taxon can easily be distinguished from *L. arayai*, *L. dringi*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana*, *L. sarawakensis*, and *L. sola* (vs. absent in the latter). By having a moderate body size (SVL 27.8–31.4 mm in males), the new species differs from *L. baluensis*, *L. brevicrus*, *L. bondangensis*, *L. fusca*, *L. itiokai*, *L. juliandringi*, *L. mjobergi*, *L. natunae*, *L. parva*, *L. palmata*, and *L. serasanae* (vs. less than 18 mm in the latter males or females).

By the moderate body size of the male (SVL 27.8–31.4 mm), the undescribed taxon differs from the smaller males *L. alpina*, *L. applebyi*, *L. ardens*, *L. aspera*, *L. aurantirosea*, *L. bashaensis*, *L. batxatensis*, *L. bidoupensis*, *L. feii*, *L. flaviglandulosa*, *L. graminicola*, *L. laui*, *L. maculosa*, *L. melica*, *L. murphyi*, *L. niveimontis*, *L. pluvialis*, *L. rowleyae*, *L. tengchongensis*, *L. verrucosa*, and *L. wumingensis* (vs. < 27 mm in the latter), and from the larger *L. albomarginata*, *L. damingshanensis*, *L. eos*, *L. nahangensis*, *L. sungi*, *L. tamdil*, and *L. zhangyapingi* (vs. larger than 32 mm in the latter).

By the presence of black spots on flanks, the undescribed taxon differs from *L. aerea*, *L. botsfordi*, *L. crocea*, *L. eos*, *L. firthi*, *L. isos*, *L. pallida*, *L. petrops*, and *L. tuberosa* (vs. absent). From the rudimentary webbing on the toes, the new species differs from *L. ardens*, *L. aurantirosea*, *L. batxatensis*, *L. duyenae*, *L. huynhi*, *L. jinshaensis*, *L. kalonensis*, *L. maculosa*, *L. oshanensis*, *L. pallida*, *L. petrops*, *L. rowleyae*, *L. shiwandashanensis*, *L. tadungensis*, *L. wuhuangmontis*, *L. wumingensis*, and *L. yachangensis* (vs. absent); from *L. sungi* and *L. tamdil* (vs. wide); and from *L. guinanensis* and *L. pelodytoides* (vs. 1/3 toe webbing). By the lateral fringes on toes being narrow, the new species differs from *L. aerea*, *L. alpine*,

Table 2. References for morphological characters of congeners of the genus *Leptobrachella*.

ID	Species	Reference
1	*L. aerea* (Rowley, Stuart, Richards, Phimmachak & Sivongxay, 2010)	Rowley et al. 2010a
2	*L. albomarginata* Wu, Yu, Kilunda, Murphy & Che, 2025	Wu et al. 2025
3	*L. alpina* (Fei, Ye & Li, 1990)	Fei et al. 1990
4	*L. applebyi* (Rowley & Cao, 2009)	Rowley and Cao 2009
5	*L. arayai* (Matsui, 1997)	Matsui 1997
6	*L. ardens* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
7	*L. aspera* Wang, Lyu, Qi & Wang, 2020	Wang et al. 2020
8	*L. aurantirosea* Ninh, Nguyen, Le, Nguyen, Quoc, Orlov, Bezman-Moseyko, Le, Nguyen & Ziegler, 2024	Ninh et al. 2024
9	*L. baluensis* Smith, 1931	Smith 1931
10	*L. bashaensis* Lyu, Dai, Wei, He, Yuan, Shi, Zhou, Ran, Kuang, Guo, Wei & Yuan, 2020	Lyu et al. 2020
11	*L. batxatensis* Hoang, Pham, Phan, Do, Wang, Jiang & Nguyen, 2025	Hoang et al. 2025
12	*L. bidoupensis* (Rowley, Le, Tran & Hoang, 2011)	Rowley et al. 2011
13	*L. bijie* Wang, Li, Li, Chen & Wang, 2019	Wang et al. 2019
14	*L. bondangensis* Eto, Matsui, Hamidy, Munir & Iskar, 2018	Eto et al. 2018
15	*L. botsfordi* (Rowley, Dau & Nguyen, 2013)	Rowley et al. 2013
16	*L. bourreti* (Dubois, 1983)	Dubois 1983
17	*L. brevicrus* Dring, 1983	Dring 1983; Eto et al. 2015
18	*L. chishuiensis* Li, Liu, Wei & Wang, 2020	Li et al. 2020
19	*L. crocea* (Rowley, Hoang, Le, Dau & Cao, 2010)	Rowley et al. 2010b
20	*L. damingshanensis* Chen, Yu, Cheng, Meng, Wei, Zhou & Lu, 2021	Chen et al. 2021c
21	*L. darongshanensis* Chen, Bei & Li, 2025	Chen et al. 2025
22	*L. dayaoshanensis* Chen, Yu, Meng & Qin, 2024	Chen et al. 2024
23	*L. dong* Liu, Shi, Li, Zhang, Xiang, Wei & Wang, 2023	Liu et al. 2023
24	*L. dorsospina* Wang, Lyu, Qi & Wang, 2020	Wang et al. 2020
	L. dushanensis Li, Li, Cheng, Liu, Wei & Wang, 2024	Li et al. 2024
25	*L. duyenae* Hoang, Pham, Phan, Do, Wang, Jiang, and Nguyen, 2025	Hoang et al. 2025
26	*L. dringi* (Dubois, 1987)	Dubois 1987
27	*L. duyenae* Hoang, Pham, Phan, Do, Wang, Jiang, and Nguyen, 2025	Hoang et al. 2025
28	*L. eos* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
29	*L. feii* Chen, Yuan & Che, 2020	Chen et al. 2020
30	*L. firthi* (Rowley, Hoang, Dau, Le & Cao, 2012)	Rowley et al. 2012
31	*L. flaviglandulosa* Chen, Wang & Che, 2020	Chen et al. 2020
32	*L. fritinniens* (Dehling & Matsui, 2013)	Dehling and Matsui 2013
33	*L. fuliginosa* (Matsui, 2006)	Matsui 2006
34	*L. fusca* Eto, Matsui, Hamidy, Munir & Isk & ar, 2018	Eto et al. 2018
35	*L. gracilis* (Günther, 1872)	Dehling 2012a
36	*L. graminicola* Nguyen, Tapley, Nguyen, Luong & Rowley, 2021	Nguyen et al. 2021
37	*L. guinanensis* Chen, Li, Peng, Liu & Huang, 2024	Chen et al. 2024
38	*L. hamidi* (Matsui, 1997)	Matsui 1997
39	*L. heteropus* (Boulenger, 1900)	Boulenger 1900
40	*L. huynhi* Hoang, Luong, Nguyen, Nguyen, Ninh, Le, Ziegler & Pham, 2024	Hoang et al. 2024
41	*L. isos* (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmett, 2015)	Rowley et al. 2015
42	*L. itiokai* Eto, Matsui & Nishikawa, 2016	Eto et al. 2016
43	*L. jinshaensis* Cheng, Shi, Li, Liu, Li & Wang, 2021	Cheng et al. 2021
44	*L. jinyunensis* Shi, Shen, Wang, Jiang & Wang, 2023	Shi et al. 2023
45	*L. juliandringi* Eto, Matsui & Nishikawa, 2015	Eto et al. 2015
46	*L. kajangensis* (Grismer, Grismer & Youmans, 2004)	Grismer et al. 2004
47	*L. kalonensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
48	*L. kecil* (Matsui, Belabut, Ahmad & Yong, 2009)	Matsui et al. 2009
49	*L. khasiorum* (Das, Tron, Rangad & Hooroo, 2010)	Das et al. 2010
50	*L. korifi* Matsui, Panha & Eto, 2023	Matsui et al. 2023
51	*L. kungfu* Zhang, Lin, Li, Su, Chen, Zhang, Zeng & Wang, 2025	Zhang et al. 2025

ID	Species	Reference
52	*L. lateralis* (Anderson, 1871)	Anderson 1871; Humtsoe et al. 2008
53	*L. laui* (Sung, Yang & Wang, 2014)	Sung et al. 2014
54	*L. liui* (Fei & Ye, 1990)	Fei et al. 1990; Sung et al. 2014
55	*L. macrops* (Duong, Do, Ngo, Nguyen & Poyarkov, 2018)	Duong et al. 2018
56	*L. maculosa* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
57	*L. mangshanensis* (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang, 2018)	Hou et al. 2018
58	*L. maoershanensis* (Yuan, Sun, Chen, Rowley & Che, 2017)	Yuan et al. 2017
59	*L. marmorata* (Matsui, Zainudin & Nishikawa, 2014)	Matsui et al. 2014b
60	*L. maura* (Inger, Lakim, Biun & Yambun, 1997)	Inger et al. 1997
61	*L. melanoleuca* (Matsui, 2006)	Matsui 2006
62	*L. melica* (Rowley, Stuart, Neang & Emmett, 2010)	Rowley et al. 2010a
63	*L. minima* (Taylor, 1962)	Ohler et al. 2011; Taylor 1962
64	*L. mjobergi* Smith, 1925	Smith 1925
65	*L. murphyi* Chen, Suwannapoom, Wu, Poyarkov, Xu, Pawangkhanant & Che, 2021	Chen et al. 2021a
66	*L. nahangensis* (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
67	*L. namdongensis* Hoang, Nguyen, Luu, Nguyen & Jiang, 2019	Hoang et al. 2019
68	*L. natunae* (Günther, 1895)	Günther 1895
69	*L. neangi* Stuart & Rowley, 2020	Stuart and Rowley 2020
70	*L. niveimontis* Chen, Poyarkov, Yuan & Che, 2020	Chen et al. 2020
71	*L. nokrekensis* (Mathew & Sen, 2010)	Mathew and Sen 2010
72	*L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
73	*L. oshanensis* (Liu, 1950)	Liu 1950; Shi et al. 2023
74	*L. pallida* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang & Nguyen, 2016)	Rowley et al. 2016
75	*L. palmata* Inger & Stuebing, 1992	Inger and Stuebing 1992
76	*L. parva* Dring, 1983	Dring 1983
77	*L. pelodytoides* (Boulenger, 1893)	Boulenger 1893; Ohler et al. 2011
78	*L. petrops* (Rowley, Dau, Hoang, Le, Cutajar & Nguyen, 2017)	Rowley et al. 2017b
79	*L. phiadenensis* Luong, Hoang, Pham, Ziegler, & Nguyen, 2023	Luong et al. 2023
80	*L. phiaoacensis* Luong, Hoang, Pham, Ziegler, & Nguyen, 2023	Luong et al. 2023
81	*L. picta* (Malkmus, 1992)	Malkmus 1992
82	*L. pingbianensis* (Rao, Hui, Zhu & Ma, 2020)	Zhu and Rao 2020
83	*L. platycephala* (Dehling, 2012)	Dehling 2012b
84	*L. pluvialis* (Ohler, Marquis, Swan & Grosjean, 2000)	Ohler et al. 2000
85	*L. puhoatensis* (Rowley, Dau & Cao, 2017)	Rowley et al. 2017a
86	*L. purpuraventra* Wang, Li, Li, Chen & Wang, 2019	Wang et al. 2019
87	*L. purpurus* (Yang, Zeng & Wang, 2018)	Yang et al. 2018
88	*L. pyrrhops* (Poyarkov, Rowley, Gogoleva, Vassilieva, Galoyan & Orlov, 2015)	Poyarkov et al. 2015
89	*L. rowleyae* (Nguyen, Poyarkov, Le, Vo, Ninh, Duong, Murphy & Sang, 2018)	Nguyen et al. 2018
90	*L. rubromammata* Poyarkov, Nguyen, Duong, Le, and Orlov, 2025	Poyarkov et al. 2025
91	*L. sabahmontana* (Matsui, Nishikawa & Yambun, 2014)	Matsui et al. 2014a
92	*L. sarawakensis* Matsui, Nishikawa, and Eto, 2025	Matsui et al. 2025
93	*L. serasanae* Dring, 1983	Dring 1983
94	*L. shangsiensis* Chen, Liao, Zhou & Mo, 2019	Chen et al. 2019
95	*L. shimentaina* Wang, Lyu & Wang, 2022	Wang et al. 2022
96	*L. shiwandashanensis* Chen, Peng, Pan, Liao, Liu & Huang, 2021	Chen et al. 2021b
97	*L. sinorensis* Matsui, Panha & Eto, 2023	Matsui et al. 2023
98	*L. sola* (Matsui, 2006)	Matsui 2006
99	*L. suiyangensis* Luo, Xiao, Gao & Zhou, 2020	Luo et al. 2020
100	*L. sungi* (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
101	*L. tadungensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
102	*L. tamdil* (Sengupta, Sailo, Lalremsanga, Das & Das, 2010)	Sengupta et al. 2010
103	*L. tengchongensis* (Yang, Wang, Chen & Rao, 2016)	Yang et al. 2018
104	*L. tuberosa* (Inger, Orlov & Darevsky, 1999)	Inger et al. 1999
105	*L. ventripunctata* (Fei, Ye & Li, 1990)	Fei et al. 1990

ID	Species	Reference
106	*L. verrucosa* Wang, Zeng, Lin & Li, 2022	Lin et al. 2022
107	*L. weixinensis* Liu, Chen, Xu & Wu, 2025	Liu et al. 2025
108	*L. wuhuangmontis* Wang, Yang & Wang, 2018	Wang et al. 2018
109	*L. wulingensis* Qian, Xiao, Cao, Xiao & Yang, 2020	Qian et al. 2020
110	*L. wumingensis* Chen, Peng, Li & Yu, 2023	Chen et al. 2023
111	*L. xishuiensis* Luo, Deng, Zhou, 2025	Luo et al. 2025
112	*L. yachangensis* Huang, Zeng, Wang, Wang, Deng, Dong, Liao & Mo, 2025	Huang et al. 2025b
113	*L. yeae* Shi, Hou, Song, Jiang & Wang, 2021	Shi et al. 2021
114	*L. yingjiangensis* (Yang, Zeng & Wang, 2018)	Yang et al. 2018
115	*L. yunkaiensis* Wang, Li, Lyu & Wang, 2018	Wang et al. 2018
116	*L. yunyangensis* Luo, Deng & Zhou, 2022	Luo et al. 2022
117	*L. yongshunensis* Huang, Wu, Jiang & Zhang, 2025	Huang et al. 2025a
118	*L. zhangyapingi* (Jiang, Yan, Suwannapoom, Chomdej & Che, 2013)	Jiang et al. 2013

L. dayaoshanensis, *L. dong*, *L. dushanensis*, *L. eos*, *L. firthi*, *L. graminicola*, *L. guinanensis*, *L. isos*, *L. khasiorum*, *L. kungfu*, *L. laui*, *L. liui*, *L. murphyi*, *L. purpurus*, *L. shimentaina*, *L. tamdil*, *L. yingjiangensis*, *L. yunkaiensis*, and *L. zhangyapingi* (vs. wide); and from *L. applebyi*, *L. ardens*, *L. crocea*, *L. darongshanensis*, *L. duyenae*, *L. kalonensis*, *L. lateralis*, *L. macrops*, *L. maculosa*, *L. melica*, *L. minima*, *L. nahangensis*, *L. namdongensis*, *L. nyx*, *L. oshanensis*, *L. pallida*, *L. pluvialis*, *L. pyrrhops*, *L. rowleyae*, *L. shiwandashanensis*, *L. tadungensis*, *L. tuberosa*, *L. wumingensis*, *L. wuhuangmontis*, and *L. xishuiensis* (vs. absent).

By the tibia–tarsal articulation reaching between eye and tympanum, the undescribed taxon differs from *L. chishuiensis* (vs. reaching the tympanum), *L. dorsospina*, *L. nokrekensis*, and *L. jinyunensis* (vs. reaching the posterior corner of eye), *L. macrops* (vs. reaching the eye), *L. korifi*, *L. neangi*, *L. suiyangensis*, and *L. yunyangensis* (vs. reaching just past the anterior edge of the eye), *L. purpuraventra* and *L. yeae* (vs. reaching the middle of eye), and *L. mangshanensis*, *L. maoershanensis*, *L. pluvialis*, *L. sinorensis*, and *L. weixinensis* (vs. reaching the nostril).

By heels not meeting when hindlimbs flexed at right angles to the axis of body, the undescribed taxon differs from *L. bijie*, *L. phiaoacensis*, and *L. phiadenensis* (vs. meeting).

By having dorsal surface shagreened with small, raised tubercles and longitudinal ridges, ventral surface creamy white with dark brown spots on chest, the undescribed taxon differs from *L. bourreti*, *L. fuliginosa*, *L. pingbianensis*, and *L. shangsiensis* (vs. dorsum smooth); from *L. ventripunctata* (vs. chest and belly with dark brown spots).

The undescribed taxon can clearly be distinguished from its phylogenetically close congeners *L. wulingensis* and *L. yongshunensis*, differs from *L. wulingensis* by heels not meeting when hindlimbs flexed at right angles to the axis of body (vs. meeting); the tibia–tarsal articulation reaching between the eye and the tympanum (vs. reaching the middle of eye); dorsal skin shagreened with small tubercles and ridge (vs. shagreened with sparse large warts, sometimes with longitudinal ridges); differs from *L. yongshunensis* by heels not meeting when hindlimbs flexed at right angles to the axis of body (vs. overlapping); the tibia–tarsal articulation reaching between the eye and the tympanum (vs. reaching the anterior edge of the eye); dorsal skin shagreened with small tubercles and ridge (vs. shagreened with sparse large warts and dense little wart grains, sometimes with longitudinal ridges.

Taxonomic accounts

Adopting an integrative taxonomic approach that combines evidence from molecular phylogenetics and detailed morphological comparisons, we conclude that the specimen represents a lineage distinct from all other congeners of *Leptobrachella*. The description of this new species is provided below.

Leptobrachella xinshaoensis sp. nov.

https://zoobank.org/FFF96E48-F1CE-4A07-8D38-8C6B9ADEAFAC

Type material. ***Holotype.*** • CIB XS20240319001 (Figs 3, 4), adult male, from Xinshao County, Hunan Province, China (27.5065°N, 111.4597°E, ca. 350 m a.s.l.), collected by Jing Liu on 19 March 2024.

Paratype. • Two male specimens, CIB XS20240319002 and CIB XS20240319003, collected on 19 March 2024 by Jing Liu from the same place as the holotype.

Etymology. The specific epithet "xinshaoensis" refers to the name of the type locality, Xinshao County, Hunan, China. We suggest the English name "Xinshao Leaf-litter Toad" and the Chinese name "Xin Shao Zhang Tu Chan (新邵掌突蟾)".

Diagnosis. *Leptobrachella xinshaoensis* sp. nov. can be distinguished from its congeners by a combination of the following characters: (1) SVL 27.8–31.4 mm in males; (2) toes rudimentary webbed, lateral fringes on toes narrow; (3) tympanum distinct, surrounded by distinct tubercles; (4) dorsal surface shagreened with small, raised tubercles and longitudinal ridges; (5) ventral surface creamy white with dark brown spots on chest and margins; (6) distinct black spots present on flanks; (7) heels not meeting when hindlimbs flexed at right angles

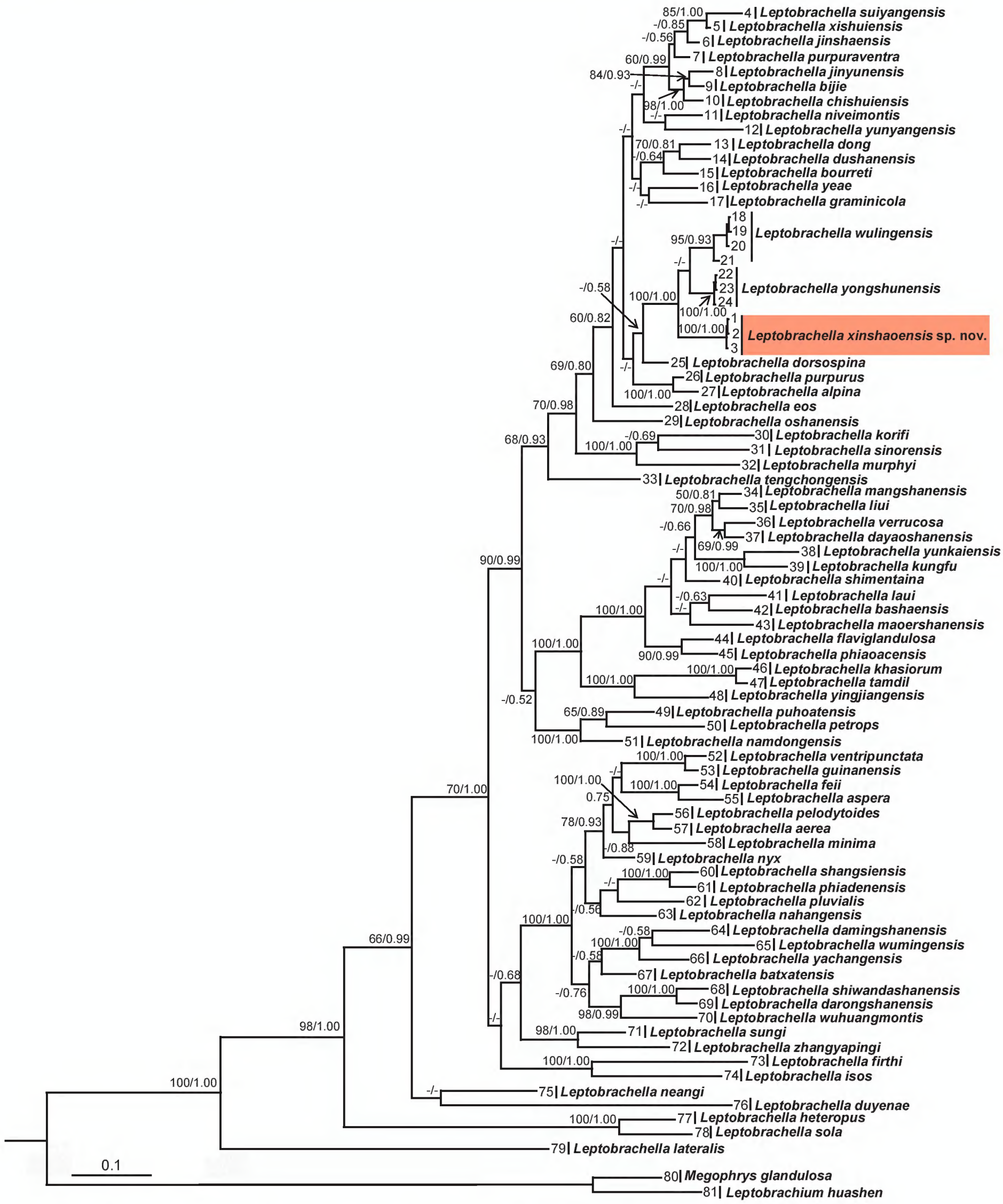

Figure 2. ML tree of the genus *Leptobrachella* reconstructed based on the 16S and COI gene sequences. Bayesian posterior probability/ML bootstrap supports are denoted beside each node, and "-" denotes BS < 50% or BPP < 0.60. Samples 1–81 refer to Table 1.

to the axis of body; (8) tibia-tarsal articulation reaching between eye and tympanum.

Description of holotype. Adult male, body size medium (SVL 31.4 mm); head length (HDL 9.6 mm) greater than head width (HDW 8.8 mm); snout rounded in ventral and lateral views, projecting slightly beyond margin of the lower jaw; nostril closer to snout than eye; loreal region particularly oblique; eye diameter (ED 3.7 mm) slightly shorter than snout length (SL 4.7 mm); eyes notably protuberant in the dorsal and lateral views, pupil vertical, copper-colored iris; tympanum distinct, rounded; tympanum diameter (TD 2.1 mm), beyond half the eye diameter, upper margin in contact with supratympanic ridge; tongue notched behind; vomerine teeth absent; su-

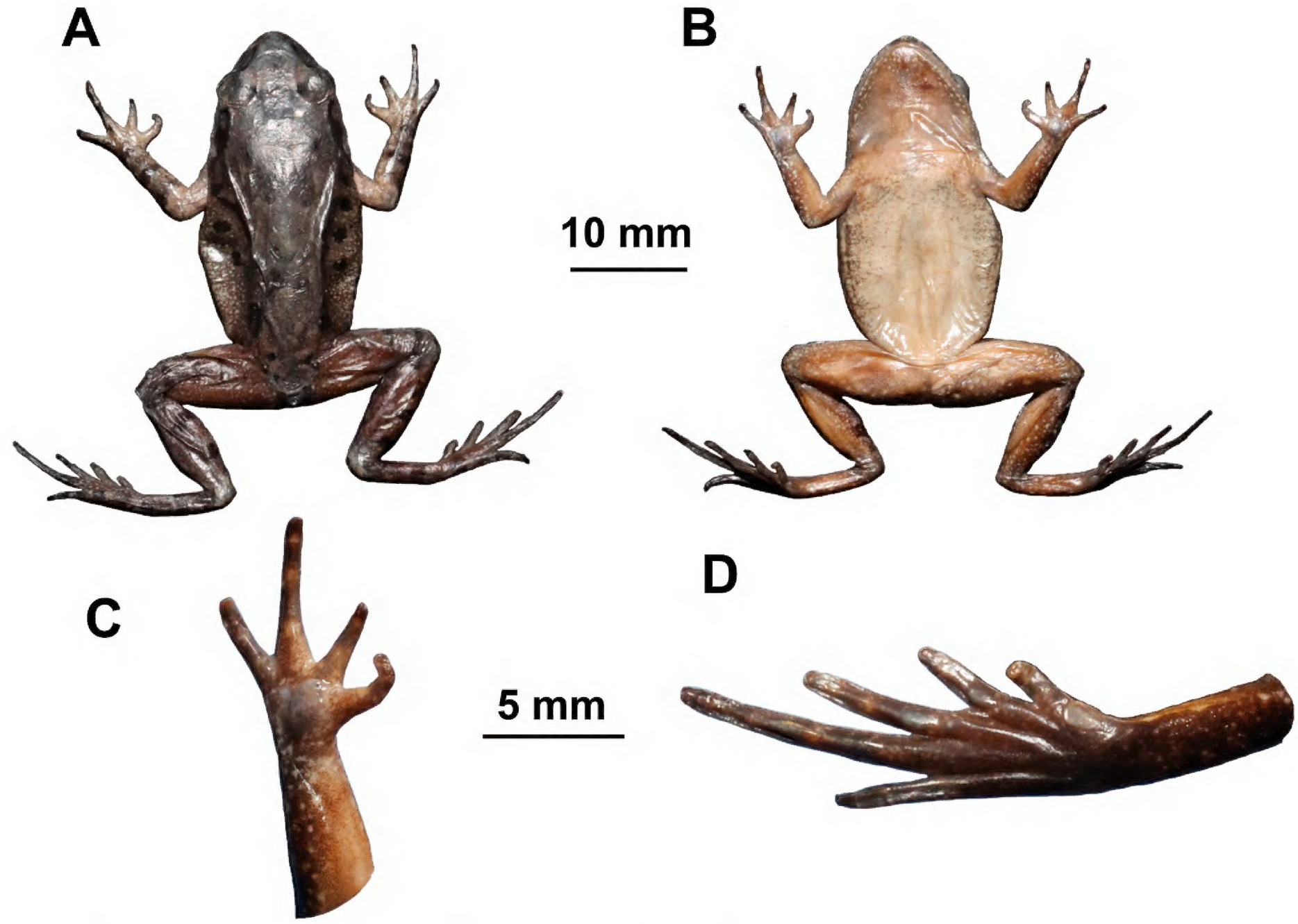

Figure 3. The holotype specimen CIB XS20240319001 of *Leptobrachella xinshaoensis* sp. nov. (preserved) in **A.** Dorsal view; **B.** Ventral view; **C.** Ventral view of hand; **D.** Ventral view of foot. (Photos **A–D** by Jing Liu.)

pratympanic ridge distinct, extending from the posterior corner of the eye to the supra-axillary gland.

Forelimb slender, length of lower arm and hand slightly shorter than half of the snout-vent length (LAL/SVL= 0.47); relative finger length I < II < IV < III; subarticular tubercles absent on fingers; supernumerary tubercles absent; finger webbing absent; lateral fringes absent; a large, round inner metacarpal tubercle, distinctly separated from small, laterally compressed outer metacarpal tubercle (Figs 3, 4).

Hindlimbs long, 1.35 times the snout-vent length; tibiotarsal articulation reaching between eye and tympanum when hindlimb is stretched along the side of the body; heels not meeting when hindlimbs are flexed and held perpendicular to body; tips of toes rounded, slightly swollen; relative length of toes: IV > III > V > II > I; subarticular tubercles indistinct under the base of II and III toe; narrow lateral fringes present on all toes; rudimentary webbing between toes; inner metatarsal tubercle distinct and prolonged, outer metatarsal tubercle absent (Figs 3, 4).

The dorsum bears sparse large warts, dense fine wart granules, and ridges; the upper eyelids, tympanic region, and dorsal surfaces of limbs are densely covered with small wart granules; the flanks possess large glandular warts; the forearms and tibiotarsal joint surfaces exhibit wart granules fused into longitudinal ridges; the inner side of the shank and the perianal region bear white conical spines; the pectoral and femoral glands are oval; the femoral glands are located on the posteroventral surface of the thigh, closer to the knee than to the vent; the supra-axillary gland is raised; the ventrolateral glandular line is distinctly visible.

Coloration of holotype in life. The dorsal surface is brown with rough skin bearing sparse, large warts. An inverted brown triangular marking is present between the eyes, followed by a "W"-shaped marking on the anterior mid-dorsum. The flanks exhibit black spots interspersed with two rows of large warts. The supra-axillary gland is orange, while the pectoral gland is indistinct. The femoral gland is small and white. The upper part of the forelimbs is orange, and the lower part displays black transverse bars. The hindlimbs feature black transverse bars, with rough skin and raised skin folds on the dorsal surface of the thighs. Both fingers and toes exhibit transverse bars. The ventral surface is creamy white, with indistinct brown markings on the lateral abdomen and chest. The jaw and throat are pinkish, with small white spots scattered along the lower lip margin. The inner thighs are pinkish with small white warts, while the outer thighs bear brown spots.

Coloration of holotype in preservation. After three months in 75% ethanol, the dorsal background color had faded to dark brown; dark vertical bars, transverse bars, and black spots remained distinctly visible. The orange pigmentation on tubercles, glands, and elbows had com-

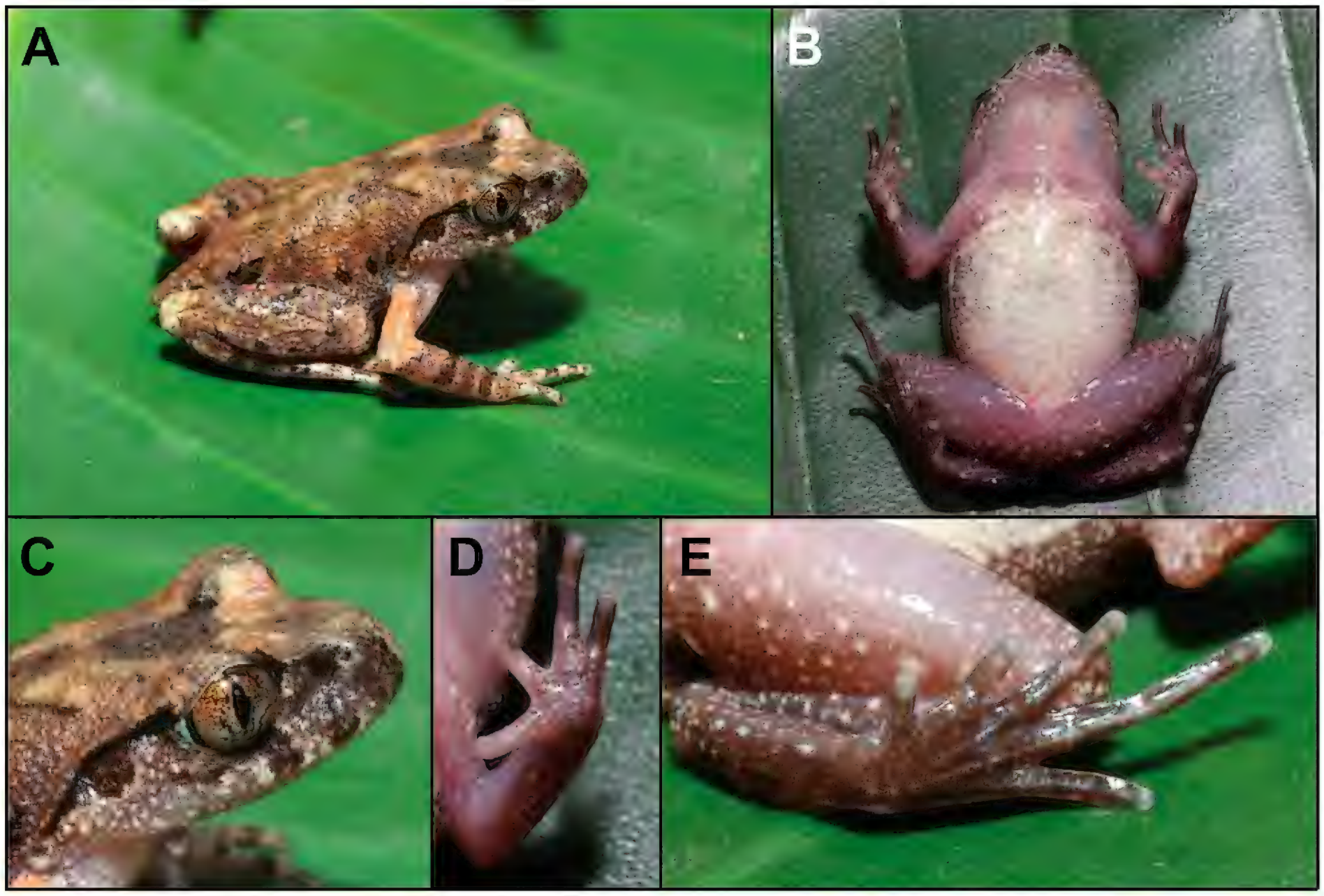

Figure 4. Photographs of the holotype CIB XS20240319001 of *Leptobrachella xinshaoensis* sp. nov. in life. **A.** Dorsal view; **B.** Ventral view; **C.** Dorsal view of head; **D.** Ventral view of hand; **E.** Ventral view of foot. (Photos **A–E** by Jing Liu.)

Table 3. Measurements of the three adult male specimens of *Leptobrachella xinshaoensis* sp. nov. Units are given in mm. See abbreviations for the morphological characters in the Materials and methods section.

Character	CIB XS20240319001	CIB XS20240319002	CIB XS20240319003	Range	Mean ± SD
ED	3.7	3.5	2.9	2.9–3.7	3.4 ± 0.4
FL	12.6	12.1	11.7	11.7–12.6	12.1 ± 0.5
HDL	10.6	9.8	9.5	9.5–10.6	10.0 ± 0.6
HDW	10.3	9.1	8.7	8.7–10.3	9.4 ± 0.8
HLL	42.4	42.0	41.7	41.7–42.4	42.0 ± 0.3
IND	4.0	3.6	3.3	3.3–4.0	3.6 ± 0.4
IOD	3.2	3.0	3.0	3.0–3.2	3.1 ± 0.1
LAL	14.8	14.2	13.9	13.9–14.8	14.3 ± 0.5
LW	2.3	2.1	1.9	1.9–2.3	2.1 ± 0.2
ML	7.7	7.2	6.9	6.9–7.7	7.2 ± 0.4
TFL	20.3	19.8	19.4	19.4–20.3	19.8 ± 0.4
SL	4.7	4.4	3.9	3.9–4.7	4.3 ± 0.4
SVL	31.4	29.6	27.8	27.8–31.4	29.6 ± 1.8
THL	12.6	11.9	11.6	11.6–12.6	12.1 ± 0.5
TL	13.1	12.8	12.2	12.2–13.1	12.7 ± 0.5
TW	3.0	2.6	2.2	2.2–3.0	2.6 ± 0.4
TYD	2.1	2.2	2.5	2.1–2.5	2.3 ± 0.2
UEW	3.0	2.9	2.3	2.3–3.0	2.7 ± 0.3

pletely faded. The ventral surfaces of the limbs appeared light brown, with brown markings along the abdominal margin becoming more distinct, while the chin and chest had gradually transitioned to light brown. The iris was uniformly dark gray.

Variation. Measurements of the type series are summarized in Table 3. All paratypes resemble the holotype in overall morphology, except for the following variations: The dorsal surface exhibits a darker coloration (Fig. 5A, C). Additionally, specimen CIB XS20240319002

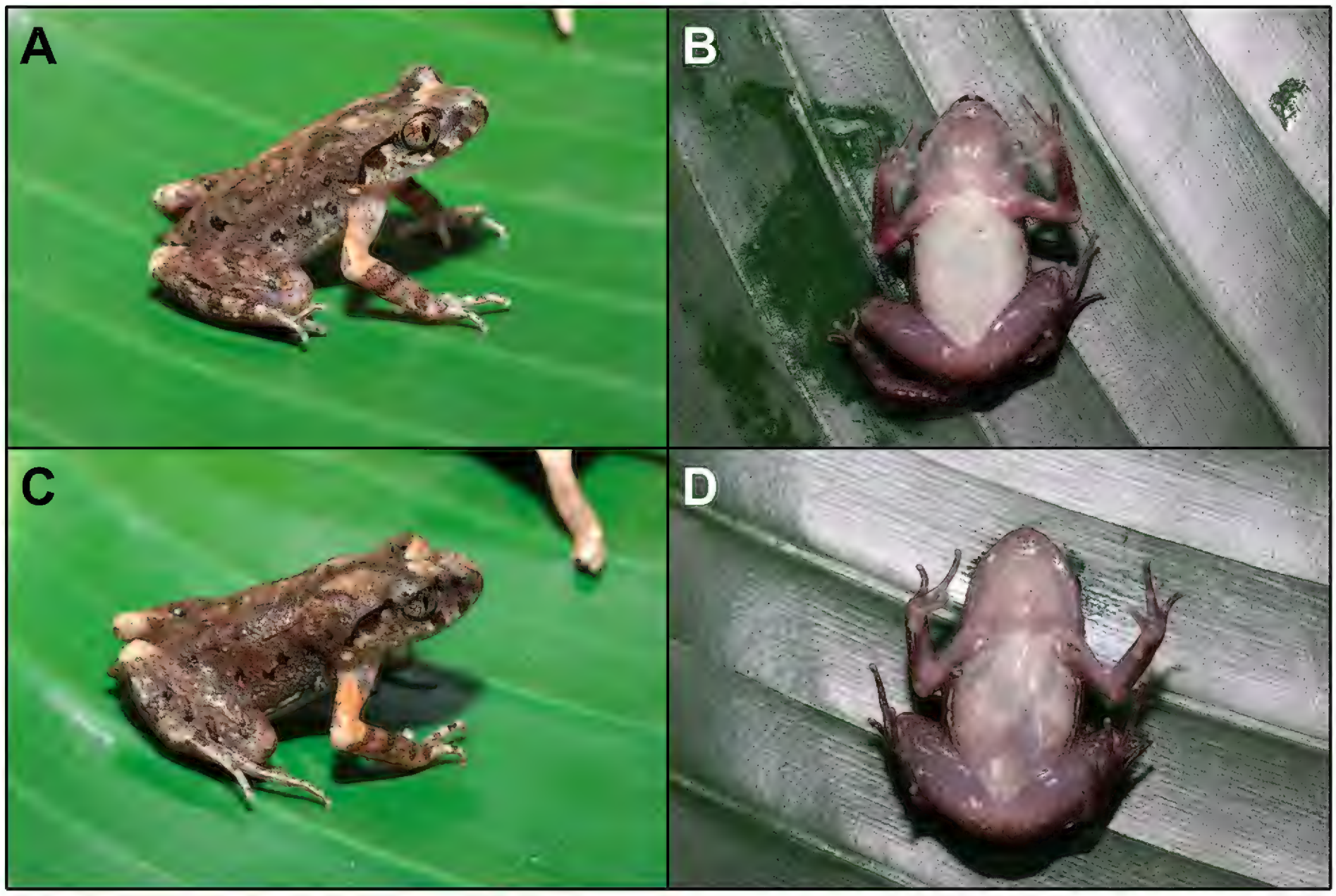

Figure 5. Color variation in *Leptobrachella xinshaoensis* sp. nov. **A.** Dorsolateral view and **B.** Ventral view of the specimen CIB XS20240319002; **C.** Dorsolateral view and **D.** Ventral view of the specimen CIB XS20240319003. (Photos **A–D** by Jing Liu.)

shows a whiter abdominal coloration (Fig. 5B), while the posterior margin of the abdomen in specimen CIB XS20240319003 is pale purple (Fig. 5D).

Secondary sexual character. Adult males with subgular vocal sacs, femoral adipose glands present on posterior surface of thigh. Nuptial pads and spines absent on males.

Distribution and habitats. *Leptobrachella xinshaoensis* sp. nov. was found on plant leaves near mountain streams with relatively torrential flow (Fig. 6). During the survey, no female individuals, eggs, or tadpoles were found, and its breeding season is speculated to be around late March.

Figure 6. Habitats of *Leptobrachella xinshaoensis* sp. nov. at the type locality, Xinshao County, Hunan Province, China. (Photo by Jing Liu.)

Discussion

Based on the integration of phylogenetic and morphological comparisons, this study reveals that the *Leptobrachella* population collected from Xinshao, Hunan, represents a previously undescribed species, which we describe herein as *Leptobrachella xinshaoensis* sp. nov. The phylogenetic tree, constructed based on the mitochondrial 16S and COI genes, strongly supports *Leptobrachella xinshaoensis* sp. nov. as an independent lineage (BPP = 1.00; BS = 100%). This clade forms a sister relationship with a clade comprising *L. wulingensis* and *L. yongshunensis*, with high nodal support (BPP = 0.99; BS = 84%; Fig. 2). This result indicates a close phylogenetic relationship between *Leptobrachella xinshaoensis* sp. nov. and species from the Wuling Mountain range. However, significant genetic differentiation already exists between *Leptobrachella xinshaoensis* sp. nov. and its closely related congeners, *L. wulingensis* and *L. yongshunensis*. The minimum interspecific genetic distances between *Leptobrachella xinshaoensis* sp. nov. and its congeners reach 2.1% (vs. *L. wulingensis*) and 8.1% (vs. *L. yongshunensis*) for the 16S and COI genes, respectively. These values meet or exceed the levels of differentiation observed

between many recognized species pairs within the genus (e.g., *L. bijie* and *L. jinshaensis*; Suppl. materials 1, 2), providing robust molecular evidence for the distinctiveness of this new species.

Detailed morphological comparisons further confirm that *Leptobrachella xinshaoensis* sp. nov. can be clearly distinguished from its phylogenetically close relatives, *L. wulingensis* and *L. yongshunensis*. For instance, the heels do not meet when the hindlimbs are flexed at right angles to the body in *Leptobrachella xinshaoensis* sp. nov., whereas they meet or overlap in the latter two sister species. Additionally, the dorsal skin is shagreened with predominantly small tubercles in *Leptobrachella xinshaoensis* sp. nov., in contrast to the sparse large warts present in the sister species. These consistent morphological differences, combined with the molecular evidence, collectively establish the taxonomic status of *Leptobrachella xinshaoensis* sp. nov.

Currently, only five *Leptobrachella* species have been reported in Hunan Province (Huang et al. 2025a). The discovery of *Leptobrachella xinshaoensis* sp. nov. extends the known distribution range of the genus further eastward to central Hunan. This species is currently known only from its type locality, where it inhabits plant leaves near mountain streams with relatively torrential flow. Its restricted distribution and specific microhabitat preference suggest a potentially high dependence on habitat quality and aquatic environments.

Given its currently limited known distribution, reliance on specific habitats, and the global threats faced by amphibians (Pottier et al. 2025), it is recommended that its conservation status be assessed in future evaluations. Furthermore, the discovery of *Leptobrachella xinshaoensis* sp. nov. highlights the potential of central Hunan as a biodiversity hotspot (Xu et al. 2024), possibly harboring other undiscovered cryptic species. Therefore, we advocate for more intensive biodiversity surveys and monitoring in this region, which is of significant importance for understanding and conserving amphibian diversity in China.

Acknowledgments

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Supplementary material 1

Uncorrected *p*-distances between the *Leptobrachella* species based on the 16S gene sequences in this study

Authors: Liming Jiang, Jing Liu, Yun Huang, Hongyan Zhang, Bin Wang

Data type: xlsx

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Supplementary material 2

Uncorrected *p*-distances between the *Leptobrachella* species based on the COI gene sequences in this study

Authors: Liming Jiang, Jing Liu, Yun Huang, Hongyan Zhang, Bin Wang

Data type: xlsx

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Supplementary material 3

Selected diagnostic characters for species described herein and species in the genus *Leptobrachella* occurring north of the Isthmus of Kra

Authors: Liming Jiang, Jing Liu, Yun Huang, Hongyan Zhang, Bin Wang

Data type: xls

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